CATHARANTHUS ALKALOIDS XXXIII[1,2]. 21'-OXO-LEUROSINE FROM *CATHARANTHUS ROSEUS* (APOCYNACEAE)

ALY EL-SAYED[3], GEORGE A. HANDY and GEOFFREY A. CORDELL

Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612

ABSTRACT.—The isolation and structure elucidation of 21'-oxo-leurosine from an alkaloid fraction of *Catharanthus roseus* is described.

More indole alkaloids have been isolated from the Madagascan periwinkle *Catharanthus roseus* (L.) G. Don than any other plant (2). This interest was generated by the isolation of several bisindole alkaloids displaying anticancer activity (2), two of which, vincaleukoblastine (VLB) and leurocristine (VCR), subsequently became clinical entities (3).

These alkaloids are not without their problems, however, and two are of some significance. First, these alkaloids are minor constituents of the plant; the typical yield of leurocristine is $3\times10^{-4}\%$ (2). Second, these alkaloids display a number of toxic side effects at therapeutic doses, the most important of which is parathesias for VCR (3). Thus, in the mid-1960's, efforts to synthesize the two component halves of VLB, 16β-carbomethoxy-velbanamine (4) and vindoline (11–14), were undertaken with a view to coupling these (or related) units in order to provide a synthesis of the bisindole species. These vast synthetic efforts culminated recently (15) in the total synthesis of VLB.

A second approach, which we have adopted, is to further investigate the extremely complex alkaloid fractions of *C. roseus* to obtain new active, but less toxic, compounds which could possibly give some direction to the continuing synthesis efforts. We report here on one of several new compounds which we have isolated and characterized.

In the process of isolating some leurosine (**1**) for the partial synthesis of another bisindole alkaloid (16), we obtained and characterized a new compound to which we have assigned the structure 21'-oxo-leurosine (**2**).

EXPERIMENTAL[4]

SEPARATION OF THE ALKALOID FRACTIONS.—The source, identification, and processing of the leaves of *Catharanthus roseus* have been discussed previously (17).

[1]For part XXXII, see ref. 1.

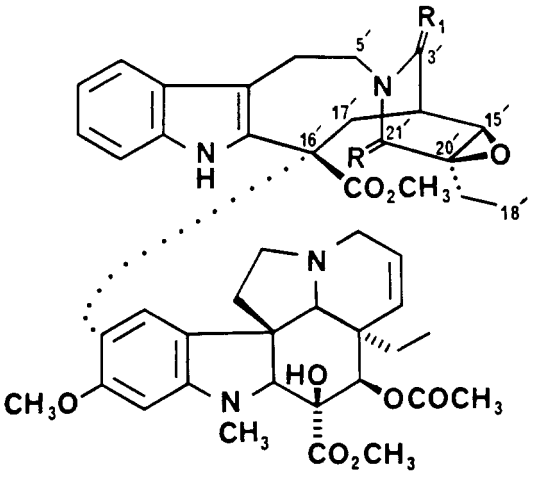
[2]These results were presented in part at the 20th Annual Meeting of the American Society of Pharmacognosy, held at Purdue University, July 29–August 3, 1979.

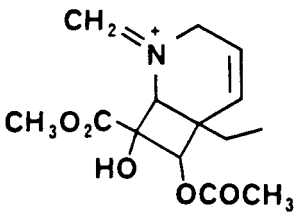
[3]Present address, Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy, University of Houston, Houston, Texas 77004.

[4]Melting points were determined by means of a Kofler hot plate and are uncorrected. The uvs pectra were obtained with a Beckman model DB-G grating spectrophotometer. The ir spectra were determined with a Beckman, model IR 18A, spectrophotometer. Proton nmr spectra were recorded in $CDCl_3$ with an IEF spectrometer operating at 240 MHz and carbon nmr spectra were recorded in $CDCl_3$ with a Bruker WP-90 at 22.68 MHz. Tetramethyl silane was used as an internal standard and chemical shifts are reported in δ units. Optical rotations were measured with a Carl Zeiss optical polarimeter. The CD spectrum was recorded on a JASCO model J-40A automatic recording spectrophotometer.

Low resolution mass spectra were obtained with a Varian MAT 112S instrument operating at 70 ev. High resolution mass spectra were obtained with an AEI MS 902 double-focusing spectrometer operating at 70 ev. Rf values were obtained by chromatography on silica gel.[5]

SEPARATION OF THE CRUDE LEUROSINE-CONTAINING FRACTION.—An aliquot (2 gm) of the crude leurosine-containing fraction was applied in chloroform to a column of silica gel PF-254[5] (100 gm) packed in chloroform. Elution with chloroform and crystallization of the residue from the first 100 ml fraction from methanol afforded white needles of **2** (79 mg) having the following physical and spectroscopic properties: mp 212–215°; $[\alpha]^{25}D$ +55.0 (c 0.10 $CHCl_3$); ir, νmax ($CHCl_3$) 3480 (m, NH and OH), 3010 (s), 1745 (s, ester CO), 1655 (m, amide CO), 1510 (m), 1435 (m), 1220 (s), 930 (m), 760 (s), 670 (s, 1,2-disubstituted benzene) cm^{-1}; uv, λmax (EtOH) (log ε) 220 (4.79) 270 (4.26) and 290 nm (4.22); NMR, δ ($CDCl_3$) 0.77 (t, J=7 Hz, 3H, 18–CH_3), 0.92 (t, J=7 Hz, 3H, 18'–CH_3), 2.03 (s, 3H, 17–$OCOCH_3$), 2.60 (s,3H, –NCH_3), 3.56 (s, 3H, 16'–CO_2CH_3), 3.67 (s, 6H, 16–CO_2CH_3 and 11–OCH_3), 4.66 (ddd, J=12,4,4 Hz, 1H, 5'–H), 5.26





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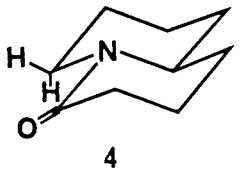

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1 R = R_1 = H_2

2 R = O; R_1 = H_2

5 R = H_2; R_1 = O

(d, J=10 Hz, 1H, 14–H), 5.44 (s, 1H, 17–H), 5.84 (ddd, J=10,4,4 Hz, 1H, 15–H), 6.11 (s, 1H, 12–H), 6.57 (s, 1H, 9–H), 7.12 (m, 3H, 9',10' and 11'–H), 7.50 (d, J=8 Hz, 1H, 12'–H) and 7.90 ppm (s, 1H, indole N–H, removed with D_2O); cmr see table 1; ms, m/e 822 (M^+, 26.6), 807 (6.3), 806 (14.1), 804 (6.3), 764 (10.9), 763 (18.8), 747 (12.5), 730 (4.7), 703 (4.7), 687 (9.4), 675 (6.3), 671 (4.7), 664 (17.8), 663 (42.2), 662 (42.1), 661 (32.8), 649 (26.6), 647 (25.0), 645 (35.9), 603 (4.7), 582 (4.6), 555 (20.3), 554 (18.7), 539 (12.5), 538 (15.6), 523 (7.8), 497 (6.2), 496 (14.0), 480 (6.2), 379 (7.8), 368 (7.8), 355 (10.9), 353 (6.2), 282 (34.3), 144 (31.2), 135 (100), 122 (43.7) and 121 (26.5). Mass measurement, Obsd. 822.3837, Calcd. for $C_{46}H_{54}N_4O_{10}$ 822.3840; Rf 0.66 ($CHCl_3$: 5% MeOH) and 0.62 (EtOAc: abs. EtOH, 3:1).

STRUCTURE ELUCIDATION OF 21'-OXO-LEUROSINE (**2**).—The uv spectrum corresponded to the summation of indole and indoline chromophores typical of the vincaleukoblastine group of alkaloids (18, 19); and the ir spectrum indicated the presence of NH, hydroxy, and saturated ester functionalities. In the proton nmr spectrum characteristic (20), signals were observed for *O*-acetyl, *N*-methyl, a *cis*-substituted olefin, aromatic methoxy, and two carbomethoxy groups (see Experimental).

One-proton singlets were observed at 6.11 and 6.57 ppm, typical of shielded aromatic protons in a para relationship. This established that the point of attachment of the indole moiety was at C–10, analogous to vincaleukoblastine. The splitting patterns of the C–18 and C–18' protons indicated the two ethyl side chains were unsubstituted.

A molecular ion at m/e 822 analyzed for $C_{46}H_{54}N_4O_{10}$ indicated the isolate contained an additional oxygen atom and two less hydrogens than leurosine (**1**). In addition, the mass spectrum displayed a number of ions typical of the presence of a vindoline moiety, particularly the ion at m/e 282 (**3**) (21).

The cd spectrum of the isolate in methanol showed two intense absorption bands of opposite

[5]E. Merck, Darmstadt, Germany.

signs at +226 ($\Delta\epsilon$ 9.5) and −210 nm($\Delta\epsilon$ 9.3). This characteristic split Cotton effect is comparable with dimers of the "natural" stereochemistry at C-16' (22, 23).

It was a combination of the ^{13}C-nuclear magnetic resonance spectrum (table 1) and an interpretation of the one-proton multiplet at 4.66 ppm which was the key to the complete structure.

Assignment of vindoline as the dihydroindole portion of this alkaloid was supported by the chemical shift assignments of all the carbon resonances of this unit on comparison with leurosine and VLB (24, 25); in this way, the attachment of the indole unit to C-10 of vindoline was confirmed.

For the indole component, aromatic carbon shifts corresponding very closely with those of leurosine (**1**) were identified in addition to characteristic resonances of a carbomethoxy group (173.4 and 52.47 ppm). The aliphatic carbons of the indole unit also corresponded very closely

TABLE 1. Comparison of the ^{13}C-nuclear magnetic resonance spectrum of leurosine (**1**) and 21'-oxo-leurosine (**2**).

Carbon	Dihydroindole unit chemical shift[a]		Carbon	Indole unit Chemical shift	
	1	**2**		**1**	**2**
2	83.10	83.16	2'	130.70	131.11
3	50.14	50.31	3'	42.27	44.00
5	50.14	50.31	5'	49.76	46.65
6	44.37	44.65	6'	24.79	23.45
7	53.00	53.06	7'	116.81	115.31
8	122.90	123.35	8'	129.17	128.90
9	123.39	122.70	9'	118.11	118.06
10	120.48	120.00	10'	122.15	123.20
11	157.70	157.82	11'	118.81	119.30
12	94.05	94.33	12'	110.29	110.51
13	152.79	153.18	13'	134.62	134.62
14	124.31	124.37	14'	33.47	31.92
15	129.76	129.77	15'	60.26	61.37
16	79.43	79.38	16'	55.21	55.11
17	76.19	76.09	17'	31.70	29.43
18	8.17	8.40	18'	8.44	8.72
19	30.61	30.67	19'	27.97	29.43
20	42.48	42.54	20'	59.96	59.53
21	65.51	65.79	21'	53.92	167.85
$COOCH_3$	170.70	170.82	$COOCH_3$	174.15	173.40
$COOCH_3$	51.97	52.14	$COOCH_3$	52.19	52.47
$N\text{-}CH_3$	38.11	38.01			
$OCOCH_3$	171.40	171.46			
$OCOCH_3$	20.90	20.97			
$Ar\text{-}OCH_3$	55.64	55.80			

[a]In parts per million downfield from TMS: δ ($CDCl_3$)+76.9 ppm.

with those of leurosine (24). In particular, an epoxide function was considered to be present on the basis of the chemical shift (59.53 ppm) of a quaternary carbon atom assignable to C-20' (24). In VLB C-20' appears at 68.6 ppm, but in leurosine the corresponding carbon appears at 59.9 ppm due to incorporation in a highly strained ring system (24). The remaining oxy-carbon at 61.37 ppm was assigned to C-15' analogous to its high-field position (60.3 ppm) in leurosine. Assignment of an epoxide unit to C-15'–C-20' as in leurosine was substantiated by the magnitude of the γ-effect on the C-18' methyl relative to VLB. A 2.0 ppm reduction (from 6.7 to 8.7 ppm) in the γ-effect on C-18' in the isolate parallels the effect observed in leurosine and is in agreement with the replacement of a 20'-hydroxy group by a C-20' ether linkage.

The most substantial difference on comparison with the spectrum of leurosine was the presence of an additional carbonyl carbon at 167.85 ppm with a concomitant loss of an amino-methylene signal suggesting the presence of an amide group. An *N*-formyl group could be excluded on the basis of the proton nmr spectrum, consequently, a cyclic amide was inferred.

Previous work (24) had established that in a simple quinolizidone such as 4, the equatorial hydrogen lies in the plane of the carbonyl group and is, therefore, deshielded.

In the isolate, the equatorial proton on an aminomethylene is shifted to 4.66 ppm and displays a geminal coupling constant of 12 Hz; the axial proton appears at 2.28 ppm. It follows the carbonyl function could either be at C-21' or C-3'.

The pmr spectrum of 3'-oxo-leurosine (**5**), previously synthesized from leurosine by oxidation with iodine under basic conditions (26), exhibited a signal for the C-14' proton at 4.76 ppm quite different in multiplicity from that observed in the isolate. This compound also displayed an apparent doublet at 4.02 ppm which is absent in the spectrum of the isolate. 21'-Oxo-leurosine (**2**) was the structure originally given to a compound obtained by treatment of leurosine with oxygen in the presence of trifluoroacetic acid (26). More recently (27) the structure of this compound was revised to that of the alkaloid catharine. Comparison of the pmr spectrum of our isolate and that of catharine indicated that they were not identical.

It follows from the above discussion that C-21' is the most favorable position for the location of the amide group, and the validity of this assignment is supported by the ^{13}C-nmr chemical shift of C-5'. Inspection of the ^{13}C nmr spectrum of the model compound **4** (28) revealed that the C-8 carbon experiences a γ effect, the magnitude of which is comparable to that observed for C-5' in the isolate. Consequently, the novel structure 21'-oxo-leurosine (**2**) is proposed for the isolate.[6]

BIOLOGICAL ACTIVITY ON THE ISOLATE.—The isolate was evaluated for anti-cancer activity according to established protocols (29). No activity was observed in the dose range 0.25–6.25 mg/kg in the P-388 lymphocytic leukemia system in mice, but the alkaloid was cytotoxic (ED_{50} 0.31 μg/ml) in the KB test system *in vitro*.

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LITERATURE CITED

1. G. A. Cordell and N. R. Farnsworth, *J. Pharm. Sci.*, **65**, 366 (1976).
2. G. H. Svoboda and D. A. Blake in "The *Catharanthus* Alkaloids" (W. I. Taylor and N. R. Farnsworth, eds.), Marcel Dekker, Inc., New York, N.Y., 1975, p. 45.
3. R. C. Delanti and W. A. Creasey, in "The *Catharanthus* Alkaloids" (W. I. Taylor and N. R. Farnsworth, eds.), Marcel Dekker, Inc., New York, N.Y., 1975, p. 237.
4. For the synthesis of derivatives of 16β-carbomethoxy-velbanamine see references 5–10.
5. G. Büchi, P. Kulsa and R. L. Rosati, *J. Am. Chem. Soc.*, **90**, 2449 (1968).
6. G. Büchi, P. Kulsa, K. Ogasawara and R. L. Rosati, *J. Am. Chem. Soc.*, **92**, 999 (1970).
7. J. P. Kutney, W. J. Cretney, P. LeQuesne, B. McKague and E. Piers, *J. Am. Chem. Soc.*, **92**, 1712 (1970).
8. J. P. Kutney and F. Bylsma, *J. Am. Chem. Soc.*, **92**, 6090 (1970).
9. M. Narisada, F. Watanabe and W. Nagata, *Tetrahedron Lett.*, 3681 (1971).
10. J. P. Kutney and F. Bylsma, *Helv. Chim. Acta*, **58**, 1672 (1975).
11. M. Ando, G. Büchi and T. Ohnuma, *J. Am. Chem. Soc.*, **97**, 6880 (1975).
12. S. Takano, K. Shishido, M. Sato and K. Ogasawara, *Heterocycles*, **6**, 1699 (1977).
13. Y. Ban, Y. Sekine and T. Oishi, *Tetrahedron Lett.*, 151 (1978).
14. J. P. Kutney, U. Bunzli-Trepp, K. K. Chan, J. P. deSouza, Y. Fujise, T. Honda, K. Katsube, F. K. Klein, A. Leutwiler, S. Morehead, M. Rohr and B. R. Worth, *J. Am. Chem. Soc.*, **100**, 4220 (1978).
15. P. Mangeney, R. Zo Andriamialisoa, N. Langlois, Y. Langlois and P. Potier, *J. Am. Chem. Soc.*, **101**, 2243 (1979).
16. A. El-Sayed, G. A. Handy and G. A. Cordell, Unpublished results.
17. G. H. Aynilian, S. G. Weiss, G. A. Cordell, D. J. Abraham, F. A. Crane and N. R. Farnsworth, J. *Pharm. Sci.*, **63**, 536 (1974).
18. M. Hesse, "Indolalkaloide in Tabellen", Springer-Verlag, Berlin, Germany, 1964.
19. M. Hesse, "Indolalkaloide in Tabellen, Ergansungswerk", Springer-Verlag, Berlin, Germany, 1968.

[6]This compound is possibly identical with a chemically modified derivative of leurosine (30) and with an alkaloid obtained from *Catharanthus ovalis* (31).

20. S. S. Tafur, J. L. Occolowitz, T. K. Elzey, J. W. Paschal and D. E. Dorman, *J. Org. Chem.*, **41**, 1001 (1976).
21. D. J. Abraham and N. R. Farnsworth, *J. Pharm. Sci.*, **58**, 694 (1969).
22. P. Potier, N. Langlois, Y. Langlois and F. Fueritte, *Chem. Commun.*, 670 (1975).
23. J. P. Kutney, D. E. Gregonis, R. Imhof, I. Itoh, E. Jahngen, A. I. Scott and W. K. Chan, *J. Am. Chem. Soc.*, **97**, 5013 (1975).
24. E. Wenkert, E. W. Hagaman and B. Lal, *Helv. Chin. Acta*, **58**, 1560 (1975).
25. D. E. Dorman and J. E. Paschal, *Org. Magn. Reson.*, **8**, 413 (1976).
26. J. P. Kutney, J. Balsevich and G. H. Bokelman, *Heterocycles*, **4**, 1377 (1976).
27. J. P. Kutney, B. John and B. R. Worth, *Heterocycles*, **9**, 493 (1978).
28. A. H. Heckendrof, K. C. Mattes, C. R. Hutchinson, E. W. Hagaman and E. Wenkert, *J. Org. Chem.*, **41**, 2045 (1976).
29. R. I. Geran, N. H. Greenberg, M. M. McDonald, A. M. Schumacher and B. J. Abbott, *Cancer Chemother. Rep.*, **3**(2), 1 (19272).
30. G. L. Thompson, G. C. Paschal and R. A. Conrad, U.S. Pat. 4,122,081; *CA* **90**: 104192p (1979).
31. P. Potier *et al.*, private communication.